

Phase I Trial of Interleukin-12 Plasmid Electroporation in Patients With Metastatic Melanoma

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Department of Malecular Medicine and Gene-based immunotherapy for cancer is limited by the lack of safe, efficient, reproducible, and Center For Mokecular Delivery, College of Medicine, Linwersity of South titratable delivery methods. Direct injection of DNA into tissue, although safer than virel vectors, Florida, Yamon Fr. suffers from low gene transfer efficiency. In vivo electroporation, in preclinical models, significantly enhances gene transfer efficiency while retaining the safety advantages of plasmid DNA. 5-ibmeted December 20, 2007;

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A phase I dose escalation trial of plasmid interleukin (IL)-12 electroporation was carried out in patients with metastatic melanoma. Patients received electroporation on days 1, 5, and 8 during a single 39-day cycle, into metastatic melanome lesions with six 100-us pulses at a 1,300-V/cm electric field through a penetrating six-electrode array immediately after DNA injection. Pre- and post-treatment biopsies were obtained at defined time points for detailed histologic evaluation and determination of IL-12 protein levels.

Twenty-four patients were treated at seven dose levels, with minimal systemic toxicity. Transient pain after electroporation was the major adverse effect. Post-treatment biopsies showed plasmid dose proportional increases in IL-12 protein levels as well as marked tumor necrosis and lymphocytic infiltrate. Two (10%) of 19 patients with nonelectroporated distant lesions and no other systemic therapy showed complete regression of all metastases, whereas eight additional patients (42%) showed disease stabilization or partial response.

Conclusion

This report describes the first human trial, to our knowledge, of gene transfer utilizing in vivo DNA electroporation. The results indicated this modality to be safe, effective, reproducible, and titratable.

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INTRODUCTION

The promise of gene therapy has not been realized, in part because of the limitations of current delivery methods. Viral vectors, probably most commonly utilized for gene delivery, have had issues with host immune response, systemic toxicity, and integration into the host genome.2-4 Plasmid DNA-based vectors avoid these particular problems, but are handicapped by the lack of efficient delivery methods. 5-7 In vivo electroporation, which utilizes an electric charge to facilitate entry of macromolecules into the cell, can be a reproducible and highly efficient method to deliver plasmid DNA.8.9 Electroporation has also been used to deliver antitumor agents such as bleomycin 10,11 (electrochemotherapy). In mice, intratumoral electroporation of interleukin (IL)-12 plasmid resulted in complete tumor re-

gression rates of 80% after three cycles of treatment, 12,13 Encouragingly, 100% of cured mice were resistant to further challenge with B16,F10 melanoma cells. No comparable tumor regression was seen in athymic mice after intratumoral plasmid IL-12 electroporation arguing for a role of T-cell immune responses in rumor regression.13 No significant organ, laboratory, or symptomatic toxicity was associated with the electrically mediated delivery of plasmid (p)1L-12 in mice.14

Melanoma is the leading cause of skin cancer death,15 Metastatic melanoma is generally treated with systemic chemotherapy or immunotherapy. 16-20 Several approaches have been utilized to enhance the effectiveness of immunotherapy using gene therapy with a variety of cytokines including IL-12.19-24 This cytokine stimulates both adaptive and innate immunity,23-24 In animal melanoma

models, the administration of pIL-12 resulted in inhibition of tumor growth as well as regression of established tumors. 12,13 Clinical phase I and II trials of systemic IL-12 (rhiL-12) protein have been reported; responses were observed in melanoma and other tumors, 25-27 Systemic rhtt.-12 protein can cause significant toxicity.28 Local delivery of IL-12 seems to be less toxic, while retaining biologic activity, in preclinical studies. 8.12.13.23-30 Recently, phase I trials have been reported with direct intratumoral injection of IL-12 plasmid DNA, 31 liposome encapsulated Semliki forest virus expressing IL-12,32 and with IL-12 producing fibroblasts.33 All of these were well tolerated; however, a limitation of these trials has been undocumented efficiency of delivery and lack of durable systemic clinical responses.

We report here the first human trial to our knowledge of the delivery of a DNA plasmid designed to express a therapeutic protein by in vivo electroporation.

PATIENTS AND METHORS

Trial Design

This trial was approved by the scientific review committee, instinctional review board, and the institutional biossfety committee at the H. Lee Moffitt Cancer Center (Tampa, Fl.) as well as the National Institutes of Health Office of Biotechnology Activities and the Food and Drug Administration, Center for Biologics Evaluation and Research. The study was conducted in two segments. Patients received treatment for one cycle only, which spanned 39 days. This cycle consisted of three plasmid injection/electroporation treatments on days 1, 5, and 8, with up to four accessible lesions injected each day.

Eligible patients had pathologically documented metastatic melanoma, stages HIB/C or IV with at least two subcutaneous or cutaneous lesions accessible for electroporation. Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of no more than 2, have adequate remal, hereatic and bone marrow function (crestimine < 1.5× upper limit of normal, bilirubin and AST within normal limits, and an absolute neutrophil count > 1,500/mm2). Patients with electronic pecemakers, defibrillators, or a history of significant cardiac arrhythmia or scizure within the last 5 years were excluded from the study.

Plasmid

The plasmid pUMVC3-hIL-12-NGVL3⁵¹ was produced under Good Manufacturing Practices (GMP) conditions at the recombinant DNA production facility at the City of Hope Center for Binesedicine and Genetics (Duaste, CA) and supplied in sterile vials at a final concentration of 1.6 mg/ml. and stored at -80°C. Before use, plasmid was thawed to 4°C and diluted in sterile saline to the required concentration.

Lidocaine was applied topically to or injected around each tumor site, and all patients were offered intravenous analgesic (morphine sulfate, 1 mst) and/or auxiolytic (lozazeparn 1 mg) medications before electroporation. Plasmid solution was injected using a 25-gauge needle into the turnor nodule to a depth no greater than 3/8 inch. A sterile applicator containing six needle electrodes arranged in a Grele was inserted into the tumor and six pulses at field strength of 1,300 V/cm and pulse duration of 100 µs were applied using a Medpulser DNA EPT System Generator (Inevio Biomedical Inc., San Diego, CA). Treatments were performed on days 1, 5, and 8.

Dose Escalation

Dose escalation was performed by increasing plasmid concentration. Plasmid was dispensed at concentrations of 0.1, 0.25, 0.5, 1.0, and 1.6 mg/mL. For cohorts 1 through 5, the plasmid injection volume was calculated using the

formula P = V/4, where P is the plasmid injection volume and V is the estimated turnor volume. Turnor volume was estimated using the formula $V = ab^3/2$ where a is the longest diameter and b is the next longest diameter perpendicular to a in any dimension. Patients in cohorts 6 and 7 received a total dose of 3.8 or 5.8 mg/treatment divided among two to four tumor sites selected irrespective of tumor volume. Dose-limiting toxicity (DET) was prespecified for this study as any hernatologic toxicity of grade 3 or greater, or nonhematologic toxicity of grade 2 or greater as defined by the National Cancer Institute Common Terminology Criteria for Adverse Events, version 2.0.

Tumor Pathology, Lymphocytic Infiltrate Assessment, and II.-12 Measurement

Patients underwent fine-needle aspiration (FNA) at an accessible disease site before treatment and FNA and excisional biopsy on days 11, 22, and 39 on electroporated lesions depending on their number and size. Excisional biopsies were bisected and one half processed for pathology and the other half for cytokine analysis. FNA biopsies were used for cytokine analysis. Histopathology specimens were sectioned at 5 µm and stained with hematoxylin and cosin, and in selected specimens immunohistochemistry was performed with anti-CD3, CD4, CD8, and CD36 antibodies using the avidin-biotin method (Vectastain ABC Kit, Plite Series, Vector Laboratories, Burlingame, CA), IL-12 and IFN-y levels in FNA and excisional biopsy samples were determined by enzyme-linked immunosorbent assay (ELISA) using the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Response Evaluation

Overall response was evaluated by a modification of Response Evaluation Criteria in Solid Tumors (RECIST). 41 Progressive disease (PD) was defined by the presence of new lesions or a 20% or greater increase in the longest diameter of an existing measurable lesion, and stable disease (SD) by an increase less than 20% in the largest diameter of a given lesion with no new distant sites of disease seen. A complete response (CR) was considered to be present only if a patient had distant (nonelectroporated) sites of disease at the start of treatment and if all sites of disease regressed completely with no evidence of disease with complete radiologic, clinical, pathologic, and laboratory evaluation. Local responses (necrosis) after the electroporation treatments were graded by our study pathologist in a blinded fishion after examining the entire biopsy.

HESULES

Patient Characteristics

Twenty-four patients were enrolled onto seven cohorts (Table 1) between December 2004 and February 2007, All patients received treatment as planned, as shown in Table 1.

Adverse Events

In vivo electroporation was associated with minimal systemic toxicity. No hematologic abnormalities were observed. The most frequent adverse event related to treatment was transient pain during the electroporation procedure (13 patients had grade 1 and 1) had grade 2 pain) and bleeding around the treatment site (13 patients had grade) and 11 grade 2 hemorrhage). We found local infiltration of 1% lidocaine around tumor lesions together with lifting the lesions off the underlying soft tissues during the electroporation procedure was effective at minimizing pain. Because no DLT was noted in cohorts 1 to 5, the experimental plan was amended to add two additional cohorts, 6 and 7, where plasmid dosage was fixed at 3.8 and 5.8 mg/treatment, respectively, and divided among the tumors selected for injection. No DLT was noted at these levels, either. The maximum administered dose in the trial was 5.8 mg administered as a fixed dose.

Table 1. Patient Characteristics and Treatment Response

Conort 1		Age 35	Pt	AJCC Stage	LDM 382	IL-12 Plasmid			Rectroporation		Objective	Response
	Patient					Concentra Img/ml	tion Lesion J Volume (ml.	No.	Site	Distant Disease Sites	Civerali Response	Duration (months
	1					. 6.1	0.56	···· 3	.2º Leg	SQ, LN	PD	
	2	54	M	IVC	927	0.1	3.9	4	Truck	SQ. LN	PD	
	3	69	54	IVC	- 923	0.1	4.4	2	Trunk	SQ	.80	
2	4	55	3.8	NC.	1,974	0.25	4.98	4	Trune:	Multiple sites	PD	
	5	66	M	EV8	368	0.25	4.03	3	Trunk	Multiple sites	SD	4
	6	43	M	IVA	483	0.25	2.98	2	Trunk, arm	SO	PD	
3	7	50	3.6	HC	541	0.5	1.16	a	Trunk, arm	SQ		> 18
	8	61	M	HC	356	0.5	9,82	4	Leg on	SQ	PD	
	9	60	Mill	N/A	449	0.6	0 13	wn 4	av Trunk, am	/ SQ :	CR	> 20
4	10	68	M	PVA.	514	3	0.07	3	Trunk	SQ	SD	> 20
	11	64	F	(VC	808	•	1.2	3	1.00	SQ, LN	PD	
	12	70	M	:BC	370	§	0.96	3	Trunk	im	PD	
5	13	61	M.	NIC.	416	. 1.8	0.57	4	Arm	ww	PD	
	14	7650	F ."	SIC -	585	1.6	0.27	8.4	Leg	SO ·SE	CR	> 16
	18	83	M	IEC.	466	1.6	0.04	4	Am	50	90	- 14
6	16	56	M	#IC	400	1.6	FV	4	Trunk	SQ	80	4
	17	79	F	1883	475	1.6	FV	3	Leg	***	SD	>4
	18	56	F	SBC	584	1,6	FV	4	Leg	SO	PD	
7	19	72-	M	HIC:	507	2: 1.6	FV	2	Leg	441	PO	
	20	43	166	3153	433	1.6	FV	4	Leg	LN USE . MA		W 4
	21	26	846	IVA	358	1.6	FV	4	100	SQ		4
	22	62	84	NA	480	1.6	PV	. 2	Truebin	~ SO	SD	•
	23	85	0.8	AVS	572	1.6	FV	- 4	Leg	SO, LN	SD	>6
	24	63	146	IVC	1,380	.÷ 1.6	FV	3	Neck	Liver, lung	PD	

Plasmid Expression

Levels of measured IL-12 increased as the plasmid dose was escalated, as demonstrated in Figure 1. The highest level of expression obtained was 2,813 pg/g of tumor on a day-11 sample from cohort 5 (patient 13). Mean (standard deviation) day-11 H-12 levels were highest in cohorts 5 and 6 (1,124 ± 1,470 picogram/gram [pg/g] and 870 ± 1,216 pg/g, respectively), representing as much as an 18-fold increase over the median baseline IL-12 measurement for the entire study group (Fig 1). Mean IL-12 levels generally were lower on days 21 and 39 than day 11, but mean 11-12 levels remained higher than baseline at day 39 in cohorts 5 and 7. To evaluate IL-12 activity, interferon-y (IFN-y) levels were measured in the biopsy samples. Levels of IPN-y generally increased and peaked at days 11 and 21 (Appendix Fig A1, online only), The highest level of expression obtained was 10,383.92 pg/g of tumor on a day 21 sample from cohort 5 (patient 15). Mean day-21 IFN-y levels were highest in cohort 5 (4,195.6 ± 5,366.63 pg/g) and mean day 11 IFN-y levels were highest in cohort 7 (2,587.4 ± 4,225.43 pg/g) representing a 7- to 60-fold increase over the median baseline IFN-y levels measurement for the entire study group (Appendix Fig A1). No increased levels of IL-12 or IFN-y were observed in serum samoles.

Tumor Necrosis and Lymphocytic Infiltration

Biopsies of injected lesions were graded for percentage of tumor necrosis and degree of lymphocytic infiltrate in a blinded fashion (Table 2). Seventy-nine skin punch biopsies were examined. Tumor necrosis levels in the sample ranged from 0% to 100%. Sixty lesions (76%) were observed to have greater than 20% necrosis with 19 (24%) and 25 (23%) brain, 50% to 59% and 100% necrosis with 19 (24%) and 25 (23%) brain, 50% to 59% and 100% necrosis respectively. The lymphocytic infiltrate ranged from a sparse peritumoral infiltrate to deuse aggregates of tumor-infiltrating lymphocytes associated with tumor cell necrosis (Fig 2).

Clinical Response

There was evidence that both injected lesions and distant noninjected lesions regressed after the treatment regimen. Nineteen of the 24 patients enrolled onto this study had additional sites of disease outside the treated lesions, and these patients could therefore be evaluated for distant responses. In 10 patients (53%) there was evidence of a systemic response resulting in either stable disease or objective regression of untreated lexions. In addition, in three of these patients (15%), all of the distant lesions regressed completely in either the absence of any other systemic antitumor therapy (two patients) or after treatment with dacarbazine (one patient). Patient 9 (cohort 3) had an 8.4 mm ulcerated high mitotic rate posterior shoulder melanoma with one positive azillary lymph node. After surgery, he received 10 months of interferon alfa 2B therapy. Two months after terminating interferon aifa 2B therapy, he started developing rapidly progressing cutaneous metastasis with more than 50 nodules on his right chest and shoulder. After treatment with pH-12 delivered with electroporation, no new lesions developed, and over a period of 18 months, all lesions flattened

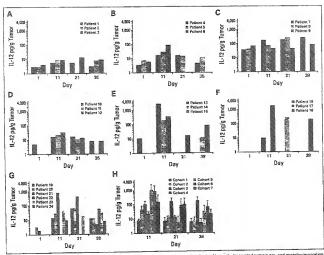


Fig 1. Interleukin (LL)-12 expression measured by enzyme-linked immunosorbent assay in samples obtained from electroporated tumors pre- and postelectroporation Each panel represents a single cohort with samples from an individual patient depicted with individual bars. The time and type of biogray specimen is as described in the work labels and the quantity of IL-12 is depicted in a logarithmic acide on the years. (A) Cohort 1, (B) cohort 2, (C) cohort 3, (D) cohort 4, (E) cohort 5, (F) cohor till a month insering and any organization of the control of the c at other cohorts have three patients.

out and faded (Fig 3A-3F). The sites of regressed lesions were biopsied at 7 and 18 months (Fig 2D), did not demonstrate evidence of melanoma, and showed only residual pigmentation, in addition, the patient had no evidence of systemic disease by positron emission tomography (PET) or computed tomography (CT) imaging at 20 months post-treatment. Patient 14 (cohort 5) had progressive cutaneous lesions in the right lower extremity (Fig 4A-4B) after multiple surgeries and hyperthermic isolated limb perfusion with melphalan. Six months after the electroporation delivery of pil. 12, the cutaneous lesions started regressing and developing hypopigmentation (halo effect) around them, and this effect persisted and the lesions have regressed further (Fig 4C-4D). A sample pigmented lesion was biopsied and showed only residual melanin pigment without any evidence of tumor. PET imaging, which had previously revealed positive results in the left calf, showed no uptake at 17 months post-treatment and continued to show no evidence of noncutaneous disease. Patient 7 (cohort 3), had an interesting post-treatment history with a rapidly progressing cutaneous metastases from a primary flank tumor that had been widely resected and irradiated after a local resection. After completing day-39 resection, the patient received dacarbazine therapy. Five months postelectroporation, after having received four cycles of dacarbazine, he had complete regression of all lesions and on a follow-up CT scan had no evidence of disease. At a further follow-up exam, now 24 months after completion of electroporation, he is radiologically and clinically free of disease. Patient 23 (cohort 7) had progressive disease in the thish and supraclavicular lymph nodes after participating in an autologous unnor vaccine trial. After pIL-12 delivery with electroporation, this patient had partial regression of local thigh lesions as well as regression of a distant supractivicular lymph node site. In six other patients, uninjected lesions remained stable, with no new lesions developing, during a period of 4 to 20 months after the end of protocol therapy (one from cohort 2, one from cohort 4, two from cohort 6, and two from cohort 7). A statistically significant correlation was

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ohort				Lesion Histology and Lymphocytic Infiltrate									
ohort				Day 11			Dev 22			Day 39			
	Patient		Necrosis (%)		Lymph	Necrosis (%)		Lymph	Necrosis (%)			Lympt	
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NOTE Lymphocytic infiltration scale. —, not measured; 0, absent; +, few lymphocytes at periphery of tumor; ++, more lymphocytes surrounding tumor; +++, ymphocytes surrounding and partially infiltrating tumor rodula; ++++, lymphocytes extensively infiltrating tumor rodule and surround infividual cells.

seen between turnor necrosis at day 39 (Table 2) and distant clinical responses (objective CR + PR + SD) by Fisher's exact test (P = .069), but no correlation was seen between lymphocyte infiltration and clinical response.

BISCUSSION.

This study evaluated the toxicity profile, tolerability, and efficacy of IL 12 plasmid delivered by electroporation. It is the culmination of several years of preclinical studies aimed at improving the effectiveness of in vivo gene transfer. Intratumoral plasmid IL-12 delivered by electroporation in the B16.F10 melanoma model can achieve local regression rates similar to those seen with electrochemotherapy or other plasmid delivery approaches, but with greater protection against tumor rechallenge, suggesting induction of a systemic antitumor immune response even in poorly immunogenic models.8,12,13 In cell culture and in animal models, electroporation greatly increases both the efficiency of gene transfer and the therapeutic efficacy of the some-based treatment, which are interrelated 6

IL-12 has been evaluated as a potential immunotherapeutic agent 22-26 Delivery of IL-12 in the form of recombinant protein caused significant toxicity. This toxicity was reduced or eliminated by delivering the IL-12 gene. 31.54-39 Comparison of efficacy across differing modalities of gene transfer in clinical trials is more difficult given the varying patient populations, small sample sizes, differing end points, and lack of quantitative expression data in these studies. De-

spite these caveats, when compared with other techniques of gene delivery, electroporation seems to produce a greater magnitude of clinical benefit in this aggressive and often fatal disease. Although intratumoral plasmid injection has resulted occasionally in local tumor response after treatment, it has only rarely resulted in regression of disease at distant sites and has not resulted in documented durable complete responses at distant sites. Local intratumoral injection of IL-12 plasmid in a recent phase I study using the same plasmid as our electroporation study resulted in local tumor regression in five of 12 patients, but no change was seen in nontreated distant lesions. 31 In this study, 11 of 12 patients had metastatic melanoma, as in our study. In another earlier trial, IL-12 plasmid was also injected directly into melanome tumors. * Four of nine patients had regression of injected tumors, and one patient had a mixed distant response but no patient experienced a distant complete response. Several other immunomodulatory gene therapy trials have been conducted in melanoma; for example, one of 51 patients on the liposomal B7-1-62 macroplobulin (Allovectio, Vical Inc. San Diego, CA) phase II trial had a distant partial regression, 29 but no patient had a distant complete regression. Similarly, in the phase I plasmid IL-2 (Leuvectin, Vical) direct-injection trial, no distant CRs were seen.40

In the current study, extensive tumor sampling with measurement of II.-12 levels, tumor histopathology, and analysis of lymphocytic infiltrate was performed. A dose-proportional increase in II-12 protein expression compared with pretreatment was seen in all patients with no significant IL-12 spillage into circulation and a correlative increase in tumor levels of IFN-y. Most (76%) electroporated

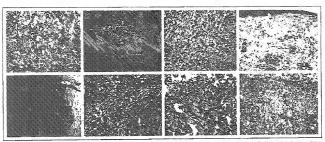


Fig. 2. Histologic appearance of electroporance lesions, An-D. Hermatonic has assolv-stained turns semiles on patient 9 (cohends 3). (M beneames tesion immediately pre-electroporation immediately and provided in a 200-XIII, Bit or dig 27 (amplication = 2004). (Co. or day 39 financiation = 2004), are (C) piginmente notate with residual provided in the provided from the cheer 15 months after the electroporation procedure was performed imagnification = 2004; (E-I) Patient 15 months after the electroporation procedure was performed imagnification = 2004; (E-I) Patient 15 months after the electroporation procedure was performed imagnification = 2004; (E-I) Patient 15 months after the electroporation procedure was performed imagnification = 2004; (E-I) Patient 15 months after the electroporation procedure was performed imagnification and object a patient with control of the electroporation and the electroporati

lesions demonstrated necrosis (> 20%) at the time of fallow-up biopsy or excision performed between 3 and 31 days after the last injection. Because IL-12 has been established to upregulate both adaptive and innate immunity, we also examined lymphocytic infibrate in

the treated tumors. Electroporated tumors demonstrated CD4*CD8* hymphocytic infiltrate in the treated lesions. The experimental regimen was found to be safe and well tolerated, with minimal systemic toxicity and with transient pain associated with the administration of

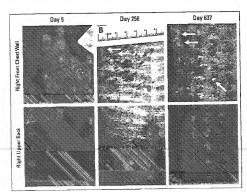


Fig. 3. Curaneous Isalons in IA-P palarity of From cabort 3 and IS-J patient 14 from cabort 3.-IA-CI Right trons these wall. (IA-P) Right uson beta. A and D were processabled of the IA-CI Right trons the IA-CI Right trons the IA-CI Right uson beta. A and D were processabled of the IA-CI Right trons the IA-CI Right trons that IA-CI Right trons the IA-CI Right trons that the electroparated leviation IS-IA-CI Right trons and IA-CI Right trons the IA-CI Right tr

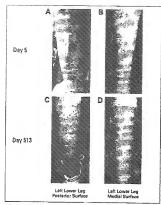


Fig. 4. Cutaneous lesions in patient 14 from othert 5. A and 5 were photographed on day 0 after the first electroporation treatment, and 0 and 0 on day \$13.50. (C) The left lower leg posterior surface. (B) The medial surface. Note the deplaymentation seen around lesions in 0 and 0.

the electrical pulse being the major adverse reaction experienced by patients.

On the basis of preclinical data, we anticipated that sugmented innate and adaptive immunity and numor necrosis at the size of treatment could result in regression of distant tumors; Pour of 19 patients who had distant disease had evidence of distant responses including three CRs in patients with progressive metastatic disease. Of these patients, two patients had not had any subsequent systemic therapy and one patient had received darathzaine after pll-12 therapy. All three CRs occurred in the setting of patients with disseminated progressive curtaneous lesions. These responses occurred over a span of 6 to 18 months with hypoplymentation and gradual volume loss occurring at sites distinct from the dectroporated sites, which argues for

immune system involvement in this effect. None of these patients have developed any new evidence of distant disease in date; in addition to these four patients, six patients had 5D lasting from 4 to 20 months at distant sites. On the base of these favorable clinical responses, a confirmatory phase It frish is blanch.

On balance, this study suggests that electroporation-mediated plasmid delivery is a powerful new tool for effective pere transfer with implications for the chinical arena. In the future, electroporation could have applications beyond the use described here in to transfer combinations of genes or knock down the expression of a given gene(s), or to produce spatially aradior temporately distinct patterns of gene expression without the safety and biohazard considerations implicit in viral vectors.

AUTHORS DISCLOSURES OF PRITENTIAL COMPLICTS OF INTEREST

Although all authors completes the dischouse destauration, the following author 6) indicated o founcial or other interest that a relevant to the subject matter under consideration in this article. Cortain relationship marked with a "U" are thous for which no compensation was received; those relationships marked with a "U" are compensated for a detailed description of the disclosure categories, or for more information about ASCOS confiller of interest policy, below refer to the Author Disclosure Declaration and the Disclosures of Potential Confilets of Interest rection in Information for Contributors.

Employment or Leadership Position; None Consultant or Advisory Roke Richard Heller, Inovio Biomedical Corp (U) Stock Ownership; Richard Heller, Inovio Biomedical Corp Honoraries None Research Fundings; None Expert Testimony; None Other Remuneration; None

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Glessary Terms

Cytokines; Cell communication molecules that are secreted in response to external stimuli.

IFN-y (interferon gamma): Cytokine that is produced by activated T cells and natural killer cells, its primary action is the activation of macrophages.

ELISA (enzyme-linked immunosorbent assay): ELISA is used to detect the presence of an antibody or an antigen in a sample.

ELISpot: Enzyme-linked immunospot that is exquisitely sensitive to assay minute amounts of mediators that are produced by cells. Typically, cells are deposited on a membrane coated with an autibody specific for a given protein. The protein of interest is captured directly around the secreting cell and is detected with an antibody specific for a different epitope. Coupled with colorimetry, the cells are visualized by specialized plate readers. Thus, the molecule is assayed before it is diluted in the supernature, captured by receptors of adjacent cells, or degraded.

Immunohistochemistry: The application of antigen-antibody interactions to histochemical techniques. Typically, a tissue section is mounted on a slide and is incubated with antibodies (polyclonal or monoclonal) specific to the antigen (primary reaction). The antigen aptibody signal is then amplified using a second antibody conjugated to a complex of peroxidase-antiperoxidase (PAP), avidin-bloxin peroxidase (ABC) or avidin-biotin alkaline phosphatase. In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen hinding. Immunofluorescence is an alternate approach to visualize antigens. In this technique, the primary antigen-antibody signal is amplified using a second antibody conjugated to a fluorochrome. On UV light absorption, the fluorochrome emits its own light at a longer wavelength (fluorescence), thus allowing localization of antibody-antigen complexes.

Plasmid: A circular, double-stranded unit of DNA that transcribes RNA within a cell independent of the chromosomal DNA.